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Quadriceps wasting and physical inactivity in patients with COPD

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ABSTRACT

Background: Quadriceps weakness is an important complication of advanced COPD but few data exist concerning muscle bulk in early disease. We hypothesised that quadriceps bulk, measured by ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$), would be reduced in mild as well as advanced COPD compared to controls and would correlate with physical activity.

Methods: 161 patients with stable COPD and 40 healthy subjects had a measurement of $USRF_{CSA}$ and wore a multisensor armband to record physical activity.

Results: $USRF_{CSA}$ was reduced in GOLD stage I patients compared to healthy subjects ($p=0.0002$). Stage II-IV patients had reduced $USRF_{CSA}$ ($p<0.0001$) compared to controls but were not significantly different from stage I disease. Physical activity level was reduced in stage I ($p=0.002$) and stage II-IV disease compared to controls. Using regression analysis, physical activity level was independently associated with $USRF_{CSA}$ in stage I ($p=0.01$), but not stage II-IV disease where residual volume to total lung capacity (RV/TLC) ratio was the only independent predictor of physical activity level.

Conclusions: Quadriceps wasting exists in patients with mild, as well as advanced, COPD and is independently associated with physical inactivity in GOLD stage I disease. The identification of these patients may guide early lifestyle and therapeutic interventions.

INTRODUCTION

Skeletal muscle dysfunction is a well recognised extrapulmonary complication of chronic obstructive pulmonary disease (COPD) with loss of lean body mass identified as a key determinant of disability [1] and an independent predictor of mortality [2]. In particular, reduced quadriceps strength is associated with reduced exercise capacity [3], impaired quality of life [4], increased healthcare use [5] and mortality independent of airflow obstruction [6].

The mechanisms involved in the development of skeletal muscle weakness in COPD are likely to be multi-factorial with systemic factors, such as oxidative stress [7], thought to interact with the key local factor of muscle inactivity [8, 9] particularly in the lower limbs [10]. Objectively measured physical activity has been identified as a strong predictor of all-cause mortality in COPD [11], highlighting its importance in a 'downward disease spiral' where progressive dyspnoea leads to reduced exercise capacity with subsequent muscle deconditioning and further inactivity [12].

Quadriceps weakness has recently been observed in the absence of severe airflow obstruction in COPD [13], and in addition there is data to suggest a reduction in physical activity in GOLD stage I patients [14]. Despite the potential rationale for muscle wasting in mild disease, little data exists on reduced quadriceps bulk in this patient group. Mid-thigh cross-sectional area measured by computed tomography (CT) has been shown to predict mortality in moderate-severe COPD [15], however the ionising radiation exposure makes this method of imaging undesirable particularly in mild disease. Magnetic resonance imaging (MRI) has also been used as a thigh

muscle imaging modality in COPD [16] but the accessibility and expense of this tool prohibit its adoption in the wider COPD population.

Ultrasound measurement of rectus femoris cross-sectional area ($USRF_{CSA}$) is a radiation-free measure of muscle bulk that relates to quadriceps strength in COPD but is effort independent [17]. We hypothesised that quadriceps wasting, measured by $USRF_{CSA}$, would be observed in mild as well as advanced COPD compared to healthy age-matched subjects and that this would correlate with daily physical activity levels.

METHODS

Patients and study design

This cross-sectional study was approved by the Joint University College London Committees on the Ethics of Human Research (Committee Alpha) and the Ethics Committee of the Royal Brompton and Harefield NHS Foundation Trust. All participants provided written informed consent. The COPD patients were recruited through outpatient clinics at the Royal Brompton Hospital, King's College and St Thomas' Hospitals as well as through public events conducted on World COPD and No-Smoking days. The period of recruitment was from August 2009 to August 2011. COPD patient diagnosis was based on NICE guidelines [18] with severity defined using GOLD stage classification. Subjects within one month of an exacerbation or with a significant co-morbidity including cardiac failure, neurological

disease or rheumatoid arthritis that limited muscle function or activity level were excluded. Healthy age-matched controls were recruited by advertisement in local newspapers.

Ultrasound Rectus Femoris Cross-sectional area

Measurement of quadriceps rectus femoris cross-sectional area was made by ultrasound using a technique previously described by Seymour *et al* [17]. B-mode ultrasonography was used with an 8MHz 7cm linear array transducer (PLF 805 ST, Toshiba Medical Systems, Crawley, UK). Two trained operators (DS and MP) performed the ultrasound examinations. The patient was positioned supine with the rested leg supported in passive extension. An anatomical landmark was found at three-fifths distance along a line from the anterior superior iliac spine to the superior patella border. The transducer was positioned in the transverse plane and orientated so that the entire rectus femoris cross-sectional area could be visualised onscreen. This image was frozen (figure 1) and the echogenic line representing the fascia around the rectus femoris was outlined manually by the operator. Rectus femoris cross-sectional area was calculated using a planimetric technique (Nemio, Toshiba Medical Systems) and the average of three consecutive measurements was taken.

Quadriceps strength and fat-free mass measurements

A volitional measurement of quadriceps maximum voluntary contraction (QMVC) was made using the technique described by Edwards *et al* 1977 [19]. Subjects sat on a modified chair with their knee fixed at 90° and performed at least 3 sustained maximal isometric quadriceps contractions. QMVC was taken as the highest tension sustained for 1 second.

Fat free mass index (FFMI) was determined by bioelectrical impedance analysis at 50kHz (BodyStat QuadScan 4000; BodyStat, Douglas, United Kingdom) and a disease specific regression equation [20]. Measurements of the impedance at 5kHz and 200kHz also allowed calculation of the bioelectrical impedance ratio (Z_{200}/Z_5). Health-related quality of life was determined using the St. George's Respiratory Questionnaire (SGRQ) and breathlessness was recorded using the Medical Research Council (MRC) dyspnoea score. A detailed description of techniques has been included in the online supplement.

Physical Activity Monitoring

Daily physical activity was recorded using a multisensor biaxial accelerometer armband (SenseWear, BodyMedia; Pittsburgh, PA) as previously described by Watz *et al* [9]. The armband incorporates physiological sensors that quantify galvanic skin response, heat flux and skin temperature to estimate energy expenditure and has been previously validated against indirect calorimetry in COPD patients [21, 22] and against the doubly labelled water technique in healthy subjects [23]. The physical activity level (PAL) was calculated using total energy expenditure (TEE) and sleep energy expenditure as a surrogate for resting energy expenditure (REE) (PAL=TEE/REE). Daily step count and PAL were measured over six consecutive days incorporating one weekend and four weekdays. A valid physical activity assessment was defined as ≥21.5 hours (90%) wearing time a day on at least 5 days. Data were downloaded and analysed using Sensewear professional software version 6.1.

Pulmonary Function Testing

Pulmonary function testing was undertaken by the Royal Brompton Hospital Lung Function Department. Spirometry, plethysmographic lung volumes, carbon monoxide diffusing capacity (TLco) (CompactLab system; Jaeger, Wurzburg, Germany) and arterial blood gases were determined in accordance with European Respiratory Society (ERS) /American Thoracic Society (ATS) recommendations [24-26].

Data and Statistical Analysis

Data are presented as mean ± SD, with accompanying p value, and analysis was performed using StatView 5.0 (Abacus concepts, Inc., Berkeley, CA, USA). Between group comparisons used analysis of variance (ANOVA), with post-hoc correction for more than 2-groups. Relationships between $USRF_{CSA}$, quadriceps strength, FFMI, impedance ratio, daily physical activity and pulmonary function were analysed using univariate and multivariate linear regression models. Figure construction was performed with GraphPad Prism Version 5.0 (GraphPad Software, San Diego, California, USA).

RESULTS

Two hundred and one subjects, comprising 161 stable COPD patients and 40 healthy age-matched volunteers, participated in the study. Baseline characteristics are shown in table 1. Of these, 154 subjects (123 COPD patients and 31 healthy participants) completed a valid physical activity assessment. Out of the remaining 47 subjects, 14 did not complete a valid period of assessment, 4 subjects declined to

participate in this part of the study and the remainder were not given an armband for logistical reasons (e.g. armband availability and subject's distance from hospital). In those subjects participating in activity monitoring, a valid period of assessment was reached in 92% (154/168). Average wearing time per day was 98% and did not significantly differ across groups (see table 2).

$USRF_{CSA}$ and quadriceps strength in COPD (Stage I-IV) and healthy subjects

$USRF_{CSA}$ and quadriceps strength (QMVC) were reduced in all GOLD stages compared to controls (table 2, figure 2 and figure 3). There were no significant differences in $USRF_{CSA}$ or QMVC across GOLD stages, except between QMVC in stage I and IV ($p<0.02$). In COPD patients, FEV_1% predicted showed no association with $USRF_{CSA}$ and a weak association with QMVC ($r=0.2$, $p=0.03$). $USRF_{CSA}$ had a linear relationship with QMVC in COPD subjects ($r=0.6$, $p<0.0001$) (online fig 1). QMVC was also significantly associated with FFMI ($r=0.54$, $p<0.0001$) and the impedance ratio (Z_{200}/Z_5) ($r=-0.54$, $p<0.0001$) in COPD (online fig 2). A multiple regression model was used to predict $USRF_{CSA}$ in all COPD subjects incorporating the significant independent variables from the univariate analysis (online table 1). Gender ($r=0.27$, $p=0.003$), QMVC ($r=0.24$, $p=0.01$), residual volume to total lung capacity (RV/TLC) ratio ($r=-0.28$, $p=0.01$), inspiratory capacity (IC) ($r=0.20$, $p=0.04$) and FFMI ($r=0.19$, $p=0.04$) were retained as independent predictors of $USRF_{CSA}$ ($r=0.75$, $p<0.0001$). In a similar multiple regression model with QMVC as the dependent variable, only $USRF_{CSA}$ ($r=0.24$, $p=0.02$) and FFMI ($r=0.25$, $p=0.01$) were retained as independent predictors of quadriceps strength in COPD ($r=0.74$, $p<0.0001$). As gender was identified as an independent variable to predict $USRF_{CSA}$,

the COPD and healthy subjects were separated into males and females (online fig 3). In both genders, $USRF_{CSA}$ was reduced in all GOLD stages compared to controls and there were no significant differences in $USRF_{CSA}$ across GOLD stages. Males (n=108) had a significantly greater $USRF_{CSA}$, compared to females (n=93), 597mm^2 vs 470mm^2, ($p<0.0001$).

Relationship of daily physical activity with GOLD stage and $USRF_{CSA}$

Daily physical activity was significantly reduced in all GOLD stages compared to healthy controls (figure 4 and figure 5). Mean group differences are shown in table 2. Daily physical activity showed a linear relationship with FEV_1% predicted (steps, r=0.6; PAL, r=0.4, $p<0.0001$) and $USRF_{CSA}$ (steps, r=0.3, p=0.002; PAL, r=0.2 $p<0.05$) in all COPD patients. In stage I disease, a multiple linear regression model to predict $USRF_{CSA}$ was used incorporating the significant independent variables from the univariate analysis (online table 2). Physical activity level was the only variable retained as an independent predictor of $USRF_{CSA}$ in stage I disease (r=0.76, p=0.01). In a similar regression analysis in stage II-IV disease, gender (r=0.29, p=0.01), RV/TLC ratio (r=−0.28, p=0.01) and IC (r=0.29, p=0.02) but not physical activity, were retained as independent predictors of $USRF_{CSA}$ (r=0.78, $p<0.0001$).

In a separate multiple linear regression model to predict physical activity in stages II-IV COPD, when incorporating the univariate correlates (online table 3), RV/TLC ratio was retained over FEV_1% predicted as the only independent variable associated with physical activity level (r=−0.23, p=0.03). Using this model in stage I COPD, $USRF_{CSA}$ but not QMVC was retained as the only independent correlate with physical activity level (r=0.64, p=0.005).

Ultrasound validity and reproducibility

A subset of 80 COPD patients had an additional mid-thigh CT scan (detailed in online supplement) with ultrasound rectus femoris cross-sectional area correlating significantly with mid-thigh CT_{CSA} ($r=0.7$, $p<0.0001$) and rectus femoris CT_{CSA} ($r=0.7$, $p<0.0001$). Further data on inter-occasion and observer variability (online fig 4), ultrasound measurement of the pennation angle and use of different measurement points are described in the supplement (online table 4).

DISCUSSION

Using $USRF_{CSA}$ we found quadriceps wasting in mild, as well as advanced COPD judged by GOLD stage. A 17% reduction in mean $USRF_{CSA}$ was observed in stage I patients compared to a healthy age matched group with a similar whole-body FFMI. The study also identified an independent association between physical activity level and $USRF_{CSA}$ in stage I disease, with this group significantly less active when compared to healthy subjects.

Significance of the findings

A recent study incorporating a large UK and Dutch COPD cohort [13] identified a 28% prevalence of quadriceps weakness in Stage I patients and supports our contention that reduced quadriceps muscle bulk is present in early disease. The simple and effort independent nature of ultrasound makes it an attractive test for detecting patients who may benefit from early intervention and avoids the need for strength measurements using research based equipment or less reliable portable

handheld devices, both of which are inherently subject to volitional influence. Interestingly, in our study, $USRF_{CSA}$ rather than quadriceps strength was independently associated with physical activity in stage I COPD, implying that this effort independent measure of quadriceps size may be a more sensitive parameter for investigating the relationship between lower limb muscle dysfunction and physical activity in patients with mild disease. This is particularly important as new COPD phenotypes are established requiring evaluation and as therapeutic interventions focus on physical activity promotion [27].

The finding of reduced daily physical activity in Stage I COPD compared to healthy subjects is supported by previous data from Watz *et al* [14] showing a reduction in activity in GOLD Stage I patients compared to a chronic bronchitis (formerly GOLD stage 0) cohort. Although their observed reduction did not reach statistical significance the comparison was not made with a healthy control group as in our current study. There have been very few other studies investigating physical activity in mild to moderate COPD patients. A multi-centre study recently found a reduction in early disease from stage II COPD onwards compared to healthy controls, however this study had a small number of patients (n=9) with GOLD stage I disease [28]. There is evidence to suggest that symptomatic GOLD stage I patients experience dynamic hyperinflation associated with dyspnoea during exercise compared to control subjects [29]. GOLD stage I patients in our study had a significantly higher MRC dyspnoea score compared to healthy controls and this may therefore provide a mechanism for the initial reduction in physical activity seen early in the disease process.

The finding of reduced physical activity in stage I COPD and its association with $USRF_{CSA}$ allows discussion of a potential mechanism for reduced quadriceps bulk in mild disease. Stage II-IV patients also demonstrated a reduction in quadriceps bulk compared to control subjects but this was not significantly different from the stage I group suggesting that a threshold level of physical inactivity, reached early in the disease process, triggers the depletion in muscle bulk. There is evidence from the Copenhagen City Heart Study [30] and elsewhere [31] that physical inactivity may in fact precede the occurrence of airflow obstruction and that it is a significant aetiological factor for the development of COPD. In addition, recent data has highlighted physical activity to be a strong predictor of all-cause mortality in COPD [11] emphasising its importance in this patient group, although that study compared activity to measures of whole body FFM and BMI, rather than quadriceps muscle bulk or strength. In keeping with previous work [14, 32], we found that lung function is associated with the level of physical activity in COPD, with RV/TLC ratio rather than FEV_1% predicted found to be an independent predictor of physical activity level in stage II-IV disease. $USRF_{CSA}$ was also independently associated with RV/TLC ratio and IC, but not FEV_1% predicted, highlighting that although FEV_1 can be used for classifying the severity of airflow obstruction [33] it does not reflect the true severity of the disease. There is increasing evidence to support measures of gas trapping and thoracic distension as better indicators of disease severity than airflow obstruction in COPD [34, 35]. Our finding that $USRF_{CSA}$ has a stronger association with physical activity in the mild compared to more advanced group, suggests that these pulmonary factors are more limiting to activity in moderate-severe patients, compared

to those with mild COPD where the association between muscle wasting and inactivity is more pronounced.

Importantly, in our study whole-body measurement of FFMI was similar in controls and patients with mild disease, although $USRF_{CSA}$ was reduced, supporting local disuse as a key factor. Disuse may also increase susceptibility to systemic factors, particularly the effects of smoking which is in itself known to be associated with skeletal muscle oxidative stress [36] and quadriceps weakness [37]. It should be noted however that inactivity may act as a significant confounder when observing quadriceps dysfunction as an effect of smoking. Further studies are needed to explore whether the fibre type switch from oxidative type I fibres to anaerobic type II fibres reported in advanced COPD [38], occurs earlier in the disease process as a consequence of physical inactivity interacting with systemic effects.

Of additional note from this study, the bioelectrical impedance ratio (Z_{200}/Z_5) has been found to show strong associations with $USRF_{CSA}$ and quadriceps strength in COPD patients; univariate correlates are shown in the supplement (online table 5). At the low (5 kHz) frequency, current does not penetrate cell membranes however at the high (200 kHz) frequency, both intracellular and extracellular spaces are penetrated. Therefore, the ratio of the bioelectrical impedance at these frequencies (Z_{200}/Z_5) is thought to give an index of separation of the two compartments; extracellular and total body water. In contrast to the use of bioelectrical impedance analysis to calculate fat free mass using regression equations which may include height, weight and gender, the impedance ratio is based on direct measurements. The ratio may be influenced by acute or chronic illness with a higher value already

shown to be associated with greater disease severity in patients with heart failure [39]. The impedance ratio therefore warrants further investigation as a non-invasive biomarker in COPD.

Critique of the method

Although this study cannot establish causation, the association between physical inactivity and depletion in muscle bulk in mild disease is strongly suggestive of a mechanistic link. The potential role of $USRF_{CSA}$ in detecting longitudinal change and response to intervention in COPD remains to be investigated.

The patients recruited in this cross-sectional study are a combination of those seen in hospital outpatients as well as those from the community setting who are not seen in secondary care (table 1). Importantly, the patients with mild disease in this study had very similar physical activity levels to the GOLD stage I cohort studied by Watz *et al* [14], suggesting that our group is representative of the general COPD population.

The strong correlation of ultrasound RF_{CSA} with both mid-thigh and rectus femoris CT measurements supports the use of rectus femoris as a representation of quadriceps bulk and confirms our initial findings in a small cohort of COPD patients [17]. Furthermore, inter-observer and inter-occasion agreement for $USRF_{CSA}$ measurement in this study were similar to that for other muscle ultrasound imaging [40]. The key areas for measurement error in our experience result from operator accuracy of probe position in relation to surface anatomy and inaccurate cursor outline of the acquired rectus femoris image, both of which may be related to

operator training and experience. CT and MR imaging modalities have been shown to have an advantage over ultrasound in serial measurements which is likely to relate to use of bony landmarks for measurement position in comparison to the use of surface anatomy. However, a randomised controlled trial using electrical muscle stimulation to reduce muscle wasting in the intensive care unit (ICU) setting has shown that ultrasound measurement of the quadriceps has strong potential as a bedside imaging modality for identifying serial changes in muscle bulk following intervention [41].

In relation to the objective measurements of activity in this study, both daily step count and PAL were used as measures of daily physical activity, although the Sensewear armband monitor has been shown to underestimate step count at slow walking speeds [22]. This may account for differences in the statistical strength of these activity variables when incorporated into the regression analyses. Importantly, the study participants showed good compliance with the Sensewear armband in keeping with recent data on the wearing time of this device in COPD and healthy subjects [42].

Conclusion

In summary, this study has shown that quadriceps wasting identified by $USRF_{CSA}$ exists in patients with mild, as well as advanced, COPD. Quadriceps bulk was associated with daily physical activity, independent of airflow limitation, in GOLD stage I disease. Our data suggest that, rather than being an end-stage phenomenon, quadriceps wasting occurs in a substantial minority of COPD patients including those with early disease. Ultrasound measurement of rectus femoris cross-sectional area

has potential as a physiological biomarker in COPD and the identification of these patients may guide early lifestyle and therapeutic interventions.

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COMPETING INTERESTS

The authors have no conflicts of interest to declare.

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FIGURE LEGENDS

Figure 1: Ultrasound assessment of the rectus femoris in the transverse plane (RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius).

Figure 2: Ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$) versus GOLD stage in COPD patients and healthy controls (ANOVA - no significant difference between I-IV). Cross bars represent the standard error of the mean (SEM).

Figure 3: Quadriceps maximal voluntary contraction (QMVC) versus GOLD stage in COPD and healthy controls (ANOVA - no significant difference between GOLD stages, except I and IV; $p<0.02$). Cross bars represent SEM.

Figure 4: Daily physical activity (steps) versus GOLD stage in COPD patients and healthy controls (ANOVA - significant differences also observed between stages 1,3 & 1,4 $p<0.0001$; 2,3 $p=0.002$; 2,4 $p<0.0001$ & 3,4 $p=0.03$). Cross bars represent SEM.

Figure 5: Physical activity level (PAL) versus GOLD stage in COPD subjects and healthy controls (ANOVA - significant differences also observed between stages 1,3 $p=0.0006$; 1,4 $p=0.0002$ & 2,4 $p=0.04$). Cross bars represent SEM.

Table 1: Baseline characteristics of COPD and control subjects

	Controls (n=40) mean (SD)	COPD I (n=38)	COPD II (n=45)	COPD III (n=41)	COPD IV (n=37)	p value
Age (years)	65 (8)	67 (9)	67 (9)	67 (9)	63 (8)	0.17
Gender (M/F)	20/20	19/19	22/23	22/19	25/12	0.45
BMI (kg/m^2)	25.9 (3.6)	26.5 (4.8)	26.6 (5.9)	25.2 (4.5)	22.4 (3.8)	0.0006
FFMI (kg/m^2)	17.8 (2.1)	17.9 (2)	17.7 (2.7)	17.6 (2.2)	16.2 (1.9)	0.004
Smoking (pack years)	9.1 (14.3)	28.1 (22.2)	42 (29.2)	50 (26.2)	55.5 (29.3)	<0.0001
Current smokers (%)	-	11	29	27	14	0.09
Outpatients (%)	-	47	60	66	89	0.001
Long-acting beta agonist (%)	-	58	80	85	100	<0.0001
Long-acting anticholinergic (%)	-	42	71	83	97	<0.0001
Inhaled corticosteroid (%)	-	58	78	83	100	<0.0001
Oral corticosteroid (% ≥5mg/day)	-	0	0	0	8	0.02
FEV_1 % pred	103.1 (11.7)	90.8 (8.8)	63.2 (8.8)	39.4 (5.5)	24 (3.8)	<0.0001
TLco% pred	89.3 (17.1)	67.5 (17.8)	58.1 (14.7)	39.9 (13.9)	26.6 (9)	<0.0001

RV%TLC ratio	34 (4.6)	40.6 (6.6)	46.6 (8)	57.9 (7.5)	64.7 (6.9)	<0.0001
IC (litres)	2.8 (0.7)	2.7 (0.8)	2.3 (0.8)	1.9 (0.6)	1.8 (0.4)	<0.0001
PaO_2 (kPa)	11.2 (1.1)	10.6 (1.5)	10.2 (1.2)	9.1 (1.2)	9.1 (1.3)	<0.0001
$PaCO_2$ (kPa)	5 (0.6)	4.7 (0.5)	5 (0.4)	5.1 (0.5)	5.4 (0.6)	<0.0001
MRC score (1-5)	1.1 (0.3)	1.8 (0.4)	2.5 (0.9)	3.1 (0.9)	3.6 (0.9)	<0.0001
SGRQ (Symptoms)	-	29.4 (24.8)	49.6 (22.8)	50 (23.7)	61.6 (19.1)	<0.0001
SGRQ (Activity)	-	34.7 (25)	58.1 (23.9)	70.1 (19.4)	84.5 (11.5)	<0.0001
SGRQ (Impacts)	-	15.5 (15.1)	29.9 (17.4)	33.8 (17.4)	48.4 (18.1)	<0.0001
SGRQ (Total)	-	23.1 (16.8)	41.1 (17.9)	47.6 (16.4)	61.5 (13.8)	<0.0001

Abbreviations: BMI - body mass index; FFMI – fat free mass index; FEV_1 - forced expiratory volume in 1 second; TLco – carbon monoxide diffusing capacity; RV – residual volume; TLC – total lung capacity; IC – inspiratory capacity; PaO_2 - arterial partial pressure of oxygen; $PaCO_2$ - arterial partial pressure of carbon dioxide; SGRQ – St George's respiratory questionnaire; Outpatients – defined as any previous hospital clinic attendance.

Table 2: Quadriceps and physical activity measurements in COPD and control subjects

	Controls mean (SD)	COPD I	COPD II	COPD III	COPD IV	P value
$USRF_{CSA}$ (mm^2)	640 (136)	530 (116)	511 (135)	504 (122)	509 (122)	<0.0001
QMVC (kg)	34.3 (8.8)	29.6 (7.2)	27.9 (7.3)	27.3 (8.8)	25.3 (6.8)	<0.0001
Step count	11735 (4399)	7960 (3430)	6606 (3328)	4010 (2316)	2219 (1157)	<0.0001
Physical Activity Level	1.69 (0.25)	1.56 (0.16)	1.47 (0.16)	1.4 (0.12)	1.38 (0.19)	<0.0001
Armband wearing time (hours/day)	23.57 (0.28)	23.60 (0.26)	23.64 (0.37)	23.57 (0.52)	23.61 (0.39)	0.95
Z_{200}/Z_5 Impedance ratio	0.789 (0.03)	0.791 (0.03)	0.806 (0.03)	0.816 (0.03)	0.814 (0.03)	0.0002

Abbreviations: $USRF_{CSA}$ – ultrasound rectus femoris cross-sectional area; QMVC – quadriceps maximal voluntary contraction.

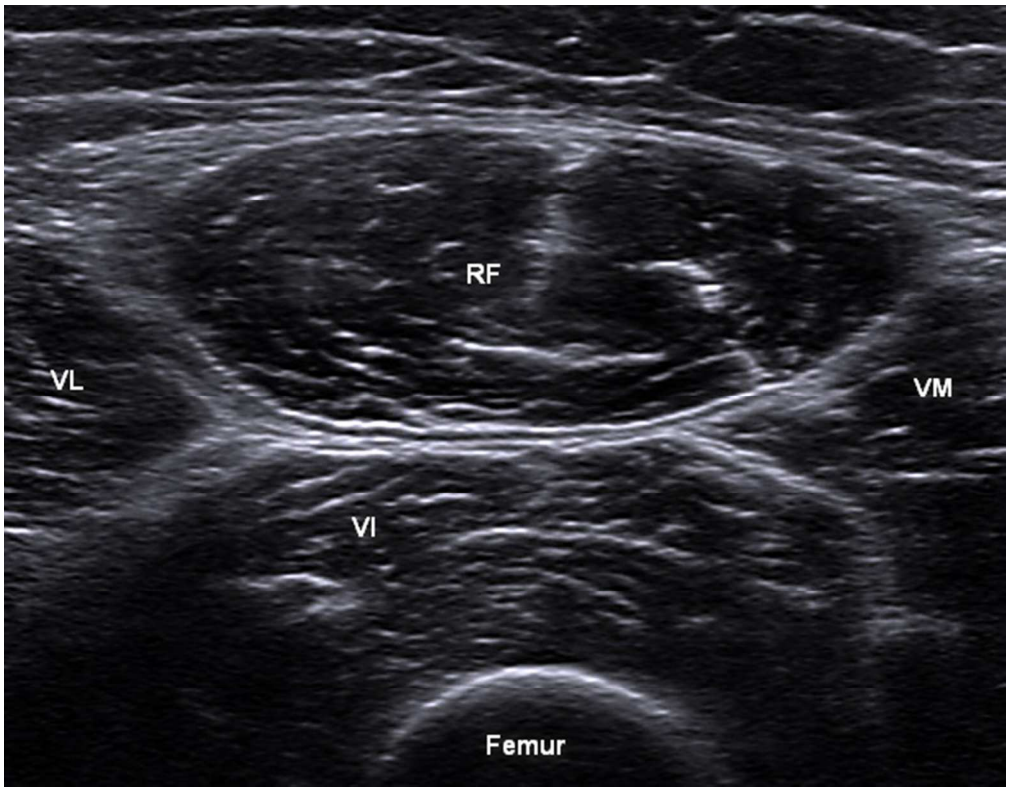


Figure 1: Ultrasound assessment of the rectus femoris in the transverse plane (RF, rectus femoris; VL, vastus lateralis; VM, vastus medialis; VI, vastus intermedius).
52x40mm (300 x 300 DPI)

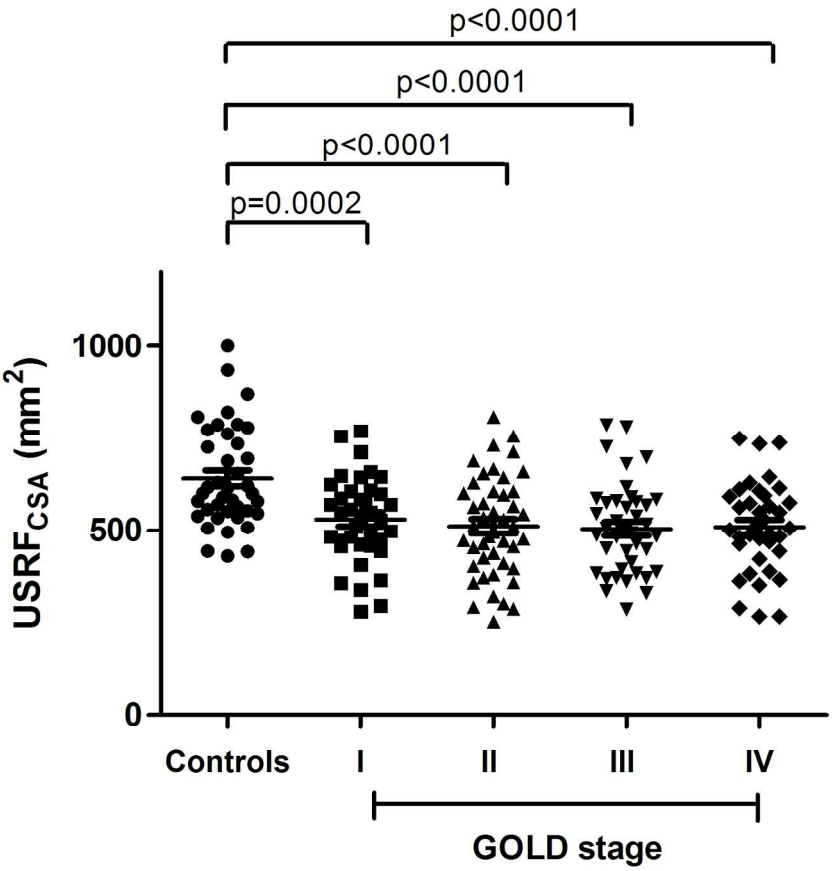


Figure 2: Ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$) versus GOLD stage in COPD patients and healthy controls (ANOVA - no significant difference between I-IV). Cross bars represent the standard error of the mean (SEM).
177x172mm (300 x 300 DPI)

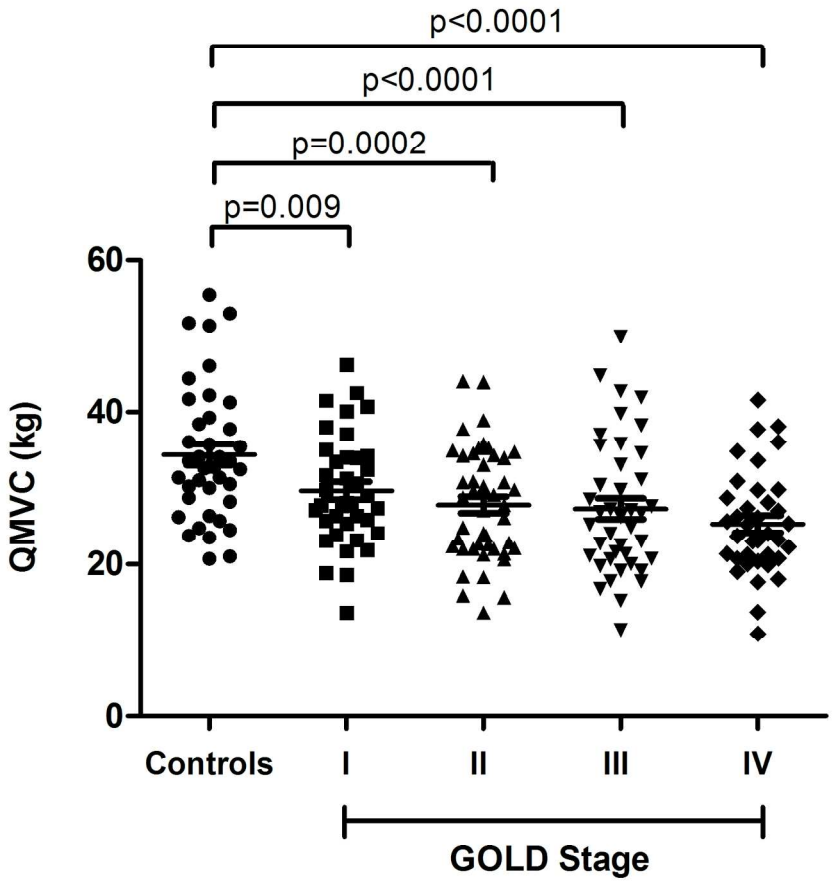


Figure 3: Quadriceps maximal voluntary contraction (QMVC) versus GOLD stage in COPD and healthy controls (ANOVA - no significant difference between GOLD stages, except I and IV; p<0.02). Cross bars represent SEM.
177x174mm (300 x 300 DPI)

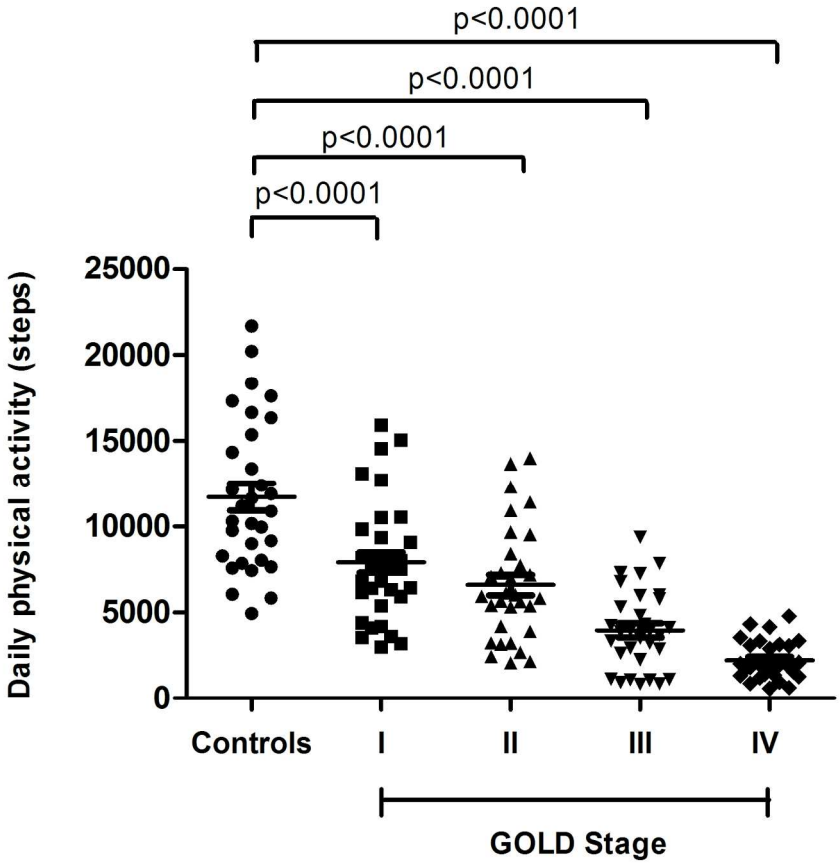


Figure 4: Daily physical activity (steps) versus GOLD stage in COPD patients and healthy controls (ANOVA - significant differences also observed between stages 1,3 & 1,4 p<0.0001; 2,3 p=0.002; 2,4 p<0.0001 & 3,4 p=0.03). Cross bars represent SEM.
177x170mm (300 x 300 DPI)

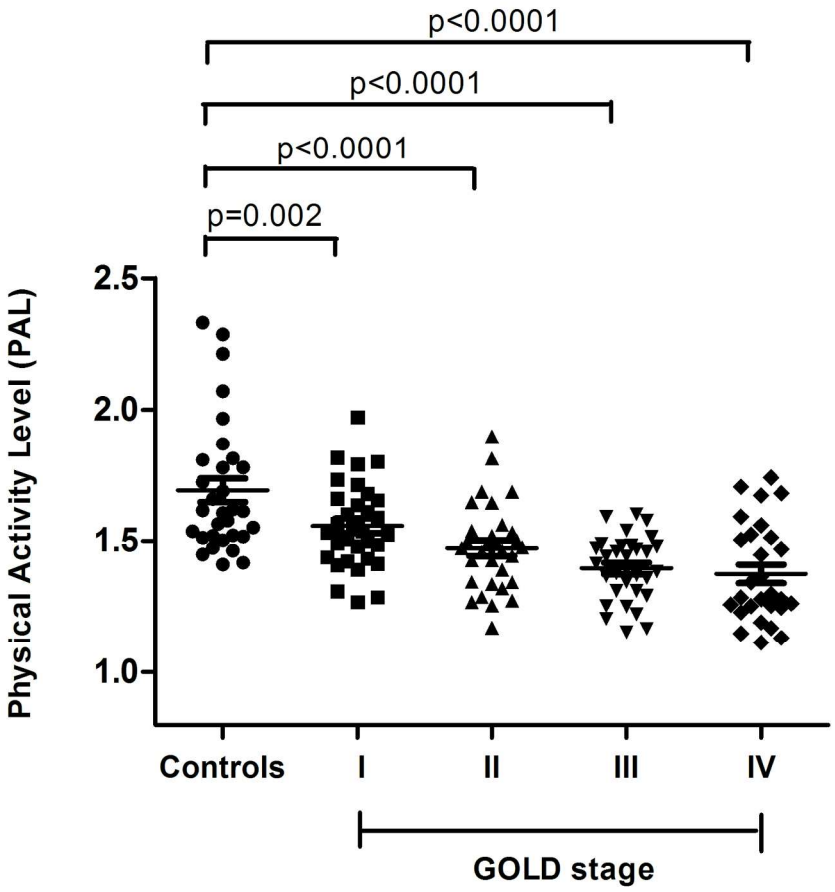


Figure 5: Physical activity level (PAL) versus GOLD stage in COPD subjects and healthy controls (ANOVA - significant differences also observed between stages 1,3 p=0.0006; 1,4 p=0.0002 & 2,4 p=0.04). Cross bars represent SEM.
177x175mm (300 x 300 DPI)

Quadriceps wasting and physical inactivity in patients with COPD

ONLINE SUPPLEMENT

Dinesh Shrikrishna, Mehul Patel, Rebecca J Tanner, John M Seymour, Bronwen A Connolly, Zudin A Puthucheary, Simon LF Walsh, Susannah A Bloch, Paul S Sidhu, Nicholas Hart, Paul R Kemp, John Moxham, Michael I Polkey, Nicholas S Hopkinson

METHODS

Quadriceps maximal voluntary contraction (QMVC)

The subject sat on a modified chair with their knee fixed at 90 degrees. An inextensible strap connected the ankle of their dominant leg to a strain gauge. The signal from the strain gauge was amplified and passed to a computer running CHART software (Labchart version 7.1, PowerLab Analogue-Digital Converter, AD instruments, Oxfordshire, UK). The subject performed at least 3 sustained maximal isometric quadriceps contractions of between 5 and 10 seconds duration. Consistent traces within 5% of maximum were obtained. A gap of approximately 30 seconds was given between each contraction to allow time to recover. Vigorous encouragement was given and the force generated was visible online using the CHART software. The QMVC was taken as highest tension sustained for 1 second.

Fat free mass measurement using bioelectrical impedance

This technique uses the electrical impedance of body tissues to determine an estimate of total body water, as electricity is conducted by dissolved ions. A two-compartment model is used which assumes that adipose tissue contains no water and that the FFM is of a particular percentage water. Single frequency, 50kHz, bioelectrical impedance values are incorporated into regression equations which include height, weight and gender to calculate fat free mass (FFM).

Mid-thigh Computed Tomography (CT) Cross-sectional area

CT was performed on a 64-slice CT scanner (Siemens SOMATOM Sensation 64, Erlangen, Germany) with the patient in a supine position. A single section of the mid-thigh at a predefined level was obtained using the following acquisition parameters:

50mAs, 120kVp. The protocol was modified to deliver a reduced amount of radiation per scan. Images were viewed and CT cross-sectional areas calculated using Digital Imaging Communications in Medicine viewing software (DicomWorks, version 1.3;*http://dicom.online.fr*) at standard window settings for visualisation of soft tissues (centre 40 HU, window width 380 HU).

RESULTS

Ultrasound rectus femoris pennation angle and measurement distance

Using values of rectus femoris pennation angle (RF_{PA}), the angle at which these muscle fibres insert into the muscle aponeurosis, to derive ultrasound rectus femoris physiological cross-sectional area ($USRF_{PCSA}$), the relationship between $USRF_{PCSA}$ and quadriceps strength (QMVC) was compared to that between anatomical $USRF_{CSA}$ alone and QMVC in a group of 31 healthy adults, 9 of whom were males, median (IQR) age 31 (24.5-37) years. $USRF_{CSA}$ and $USRF_{PCSA}$ were measured at both three fifths and two thirds distance from anterior superior iliac spine to the superior patellar border. Correlations were determined using Pearson's correlation coefficient and revealed no differences in relationship between anatomical ($USRF_{CSA}$) and physiological ($USRF_{PCSA}$) cross-sectional areas measured at either distance, and quadriceps strength, suggesting that pennation angle added no further value to the relationship between cross-sectional area and strength (online table 4).

FIGURE LEGEND

Online Figure 1: Quadriceps strength (QMVC) versus ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$) in COPD patients (Pearson correlation; r=0.6, p<0.0001)

Online Figure 2: Quadriceps strength (QMVC) versus Impedance ratio in COPD patients (Pearson correlation; r=−0.54, p<0.0001).

Online Figure 3: Ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$) in COPD patients and healthy controls separated by gender - males (A) and females (B); (ANOVA - no significant difference between stages I-IV). Cross bars represent SEM.

Online Figure 4: Bland-Altman analysis comparing the rectus femoris cross-sectional area (RF_{CSA}) measured by ultrasound on two separate occasions (n=21). Mean (SD) bias - 0.06 (0.21) cm^2 : coefficient of repeatability 0.41cm^2, Dotted line represents 95% limits of agreement -0.47 to +0.36 cm^2 , index of reliability 0.97. Similar analysis was conducted for inter-observer variability (n=10): mean (SD) bias 0.11 (0.29) cm^2 : coefficient of repeatability 0.57cm^2, 95% limits of agreement -0.46 to +0.68 cm^2, index of reliability 0.97.

TABLES

Online Table 1: Univariate correlates of $USRF_{CSA}$ in all COPD subjects

	$USRF_{CSA}$ (r)	p
Age	−0.20	0.01
Gender	0.48	<0.0001
FFMI	0.49	<0.0001
FEV_1 % pred	0.08	0.32
TLco% pred	0.26	0.001
RV/TLC ratio	−0.22	0.006
IC	0.44	<0.0001
QMVC	0.60	<0.0001
Impedance ratio	−0.53	<0.0001
PAL	0.20	<0.05
Steps	0.30	0.002

Abbreviations: QMVC - quadriceps maximum voluntary contraction; $USRF_{CSA}$ - ultrasound rectus femoris cross-sectional area; FEV_1 - forced expiratory volume in 1 second; RV – residual volume; TLC – total lung capacity; IC – inspiratory capacity; TLco – carbon monoxide diffusing capacity; FFMI - fat free mass index; PAL – physical activity level; r and p values derived from Pearson's correlation coefficient.

Online Table 2: Univariate correlates of $USRF_{CSA}$ in stage I COPD subjects

	$USRF_{CSA}$ (r)	p
Age	−0.33	0.04
Gender	0.50	0.001
FFMI	0.43	0.007
FEV_1 % pred	0.07	0.70
TLco% pred	0.38	0.03
RV/TLC ratio	−0.42	0.01
IC	0.41	0.02
QMVC	0.63	<0.0001
Impedance ratio	−0.64	<0.0001
PAL	0.70	<0.0001
Steps	0.53	0.002

Abbreviations: QMVC - quadriceps maximum voluntary contraction; $USRF_{CSA}$ - ultrasound rectus femoris cross-sectional area; FEV_1 - forced expiratory volume in 1 second; RV – residual volume; TLC – total lung capacity; IC – inspiratory capacity; TLco – carbon monoxide diffusing capacity; FFMI - fat free mass index; PAL – physical activity level. r and p values derived from Pearson's correlation coefficient.

Online Table 3: Univariate correlates of physical activity in stages II-IV COPD

	Steps (r)	p	PAL (r)	p
Age	−0.12	0.25	−0.08	0.46
Gender	0.18	0.10	0.14	0.18
FFMI	0.19	0.07	0.10	0.40
$USRF_{CSA}$	0.24	0.02	0.05	0.70
FEV_1% pred	0.57	<0.0001	0.21	<0.05
TLco% pred	0.54	<0.0001	0.07	0.50
RV/TLC ratio	−0.53	<0.0001	−0.23	0.03
IC	0.27	0.01	0.01	0.92
QMVC	0.22	0.04	0.02	0.82
Impedance ratio	−0.27	0.01	−0.12	0.30

Abbreviations: QMVC - quadriceps maximum voluntary contraction; $USRF_{CSA}$ - ultrasound rectus femoris cross-sectional area; FEV_1 - forced expiratory volume in 1 second; RV – residual volume; TLC – total lung capacity; IC – inspiratory capacity; TLco – carbon monoxide diffusing capacity; FFMI - fat free mass index; PAL – physical activity level. r and p values derived from Pearson's correlation coefficient. Using regression analysis, RV/TLC ratio was retained over FEV_1% predicted as the only independent predictor of physical activity level in stage II-IV COPD patients ($p=0.03$). FEV_1% predicted and TLco% predicted were independent predictors of step count ($p<0.0001$).

Online Table 4: Relationship between rectus femoris anatomical cross-sectional area (RF_{CSA}) and rectus femoris physiological cross-sectional area (RF_{PCSA}) with quadriceps strength

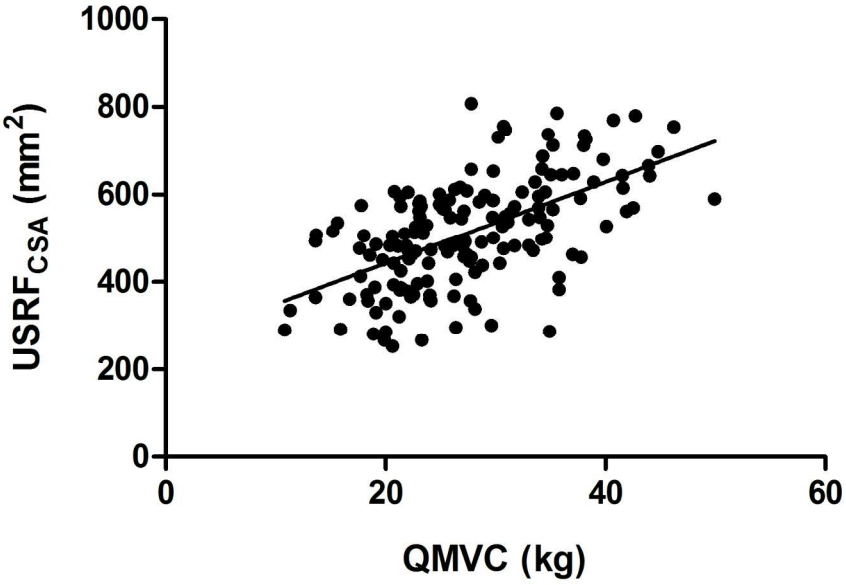
	r	p
3/5 $USRF_{CSA}$ + QMVC	0.7	0.001
3/5 $USRF_{PCSA}$ + QMVC	0.7	0.001
2/3 $USRF_{CSA}$ + QMVC	0.6	0.002
2/3 $USRF_{PCSA}$ + QMVC	0.6	0.002

Abbreviations: QMVC - quadriceps maximum voluntary contraction; $USRF_{CSA}$ - ultrasound rectus femoris cross-sectional area; $USRF_{PCSA}$ - rectus femoris physiological cross sectional area; 3/5 - measurements performed at three fifths distance between anterior superior iliac spine and superior patellar border; 2/3 - measurements performed at two thirds distance. r and p values derived from Pearson's correlation coefficient.

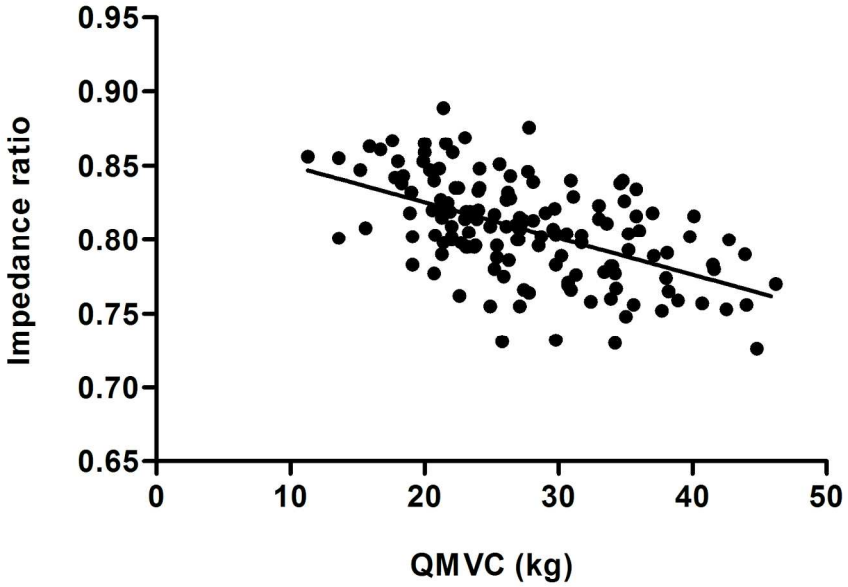
Online Table 5: Univariate correlates of the impedance ratio in COPD patients.

	r	p
Age	0.37	<0.0001
Gender	0.34	<0.0001
QMVC	−0.54	<0.0001
$USRF_{CSA}$	−0.53	<0.0001
FEV_1% pred	−0.32	0.0001
FFMI	−0.48	<0.0001
Steps	−0.35	0.0001
PAL	−0.27	0.003

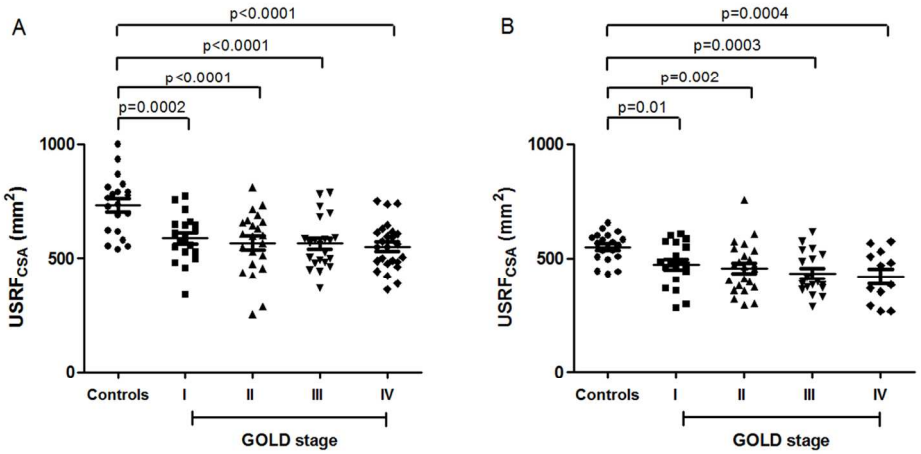
Abbreviations: QMVC - quadriceps maximum voluntary contraction; $USRF_{CSA}$ - ultrasound rectus femoris cross-sectional area; FEV_1 - forced expiratory volume in 1 second; FFMI - fat free mass index; PAL – physical activity level. r and p values derived from Pearson's correlation coefficient.



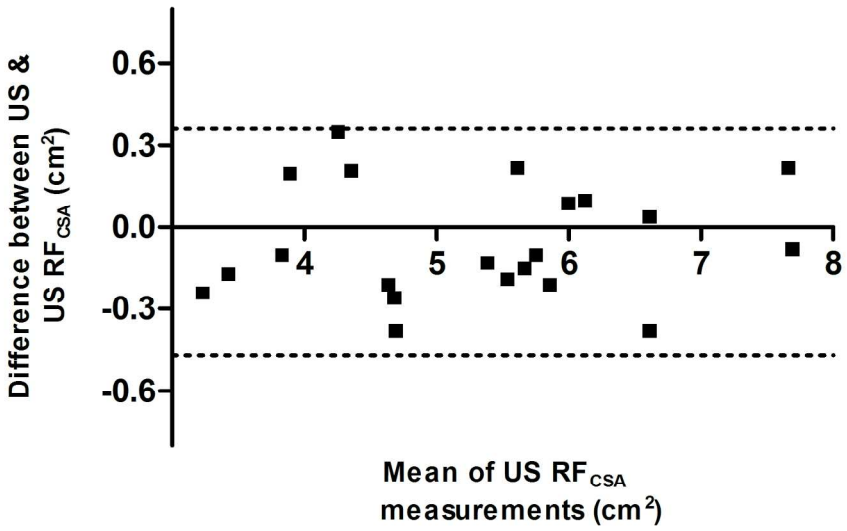
Online Figure 1: Quadriceps strength (QMVC) versus ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$) in COPD patients (Pearson correlation; r=0.6, p<0.0001)
177x123mm (300 x 300 DPI)



Online Figure 2: Quadriceps strength (QMVC) versus Impedance ratio in COPD patients (Pearson correlation; r=-0.54, p<0.0001).
177x123mm (300 x 300 DPI)



Online Figure 3: Ultrasound rectus femoris cross-sectional area ($USRF_{CSA}$) in COPD patients and healthy controls separated by gender - males (A) and females (B); (ANOVA - no significant difference between stages I-IV). Cross bars represent SEM.
253x126mm (150 x 150 DPI)



Online Figure 4: Bland-Altman analysis comparing the rectus femoris cross-sectional area (RF_{CSA}) measured by ultrasound on two separate occasions (n=21). Mean (SD) bias - 0.06 (0.21) cm^2:coefficient of repeatability 0.41cm^2, Dotted line represents 95% limits of agreement -0.47 to +0.36 cm^2 , index of reliability 0.97. Similar analysis was conducted for inter-observer variability(n=10): mean (SD) bias 0.11 (0.29) cm^2 : coefficient of repeatability 0.57cm^2, 95% limits of agreement -0.46 to +0.68 cm^2, index of reliability 0.97.
178x115mm (300 x 300 DPI)